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Abstracts

# Stem cells and tissue regeneration

## Program/Abstract # 283

### Neural stem cell erythropoietin receptor expression during human fetal brain development

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Erythropoietin receptor (EPOR) expression is necessary to promote neurogenesis. In EPOR-null mice, the average number of neuronal cells were significantly lower. Thus, EPOR expression of neural stem cells (NSC) is critical during development of fetal central nervous system (CNS). However, there is no systemic data to depict the EPOR of NSC at different gestational stages. In current study, fresh isolated NSC from fetal human brain tissues with different gestation (8–9 weeks, 10–20 wks and 23–24 wks) were used to analyze EPOR gene signal and protein expressions. NSCs isolated from 8 to 9 wks brain express RT-PCR and immunoprecipitation detectable level of EPOR. From 2nd trimester (10–20 wks) brain tissues, the EPOR expression density of NSC increased 4 to 10 and 3 to 7 folds from both RT-PCR and immunoblot assays respectively. NSCs from 20 wks and 24 wks fetal brain tissue expressed similar level of EPOR. NSCs from 1st trimester brain have minimal EPOR compared with cells from 2nd and 3rd trimester. EPOR expression gradually increased following the fetal development. To quantify NSC membrane EPOR, receptor binding study was performed with $^{125}$I-labeled erythropoietin (EPO). Approximately $4 \pm 1.2 \times 10^2$ binding sites/cell appeared from NSC isolated from 9 wks, $6.4 \pm 0.8 \times 10^3$/cell from18 wks, and $6.8 \pm 1.6 \times 10^3$/cell from 24 wks brain. The binding results confirmed receptor protein expression manners of EPOR among the NSCs from different gestation stages. These findings suggest that EPO may be significant for later brain development rather than that at 1st trimester.

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## Program/Abstract # 284

### Frozen human fibroblast cultures of varying ages contain numerous multipotent cells capable of in vitro differentiation into cells of all three germ layers

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We show that multipotent cells are found in frozen human fibroblasts cultures grown under conditions used by De Coppi et al. (2007) for amniotic fluid-derived stem cells. Cells from amniotic fluid (passage 7), fetal tissue (passage 10), a 3-day-old (passage 9), an 11-year-old (passage 9), a 37-year-old (passage 10), and a 96-year-old (passage 8) were obtained (Coriell Cell Repository). Previous studies show amniotic fluid cells at passage 8/> were highly positive for the hemopoietic stem cell marker, CD117, and easily differentiate into various cell types (Sciorra et al., 2007). Cells from the repository were grown in Eagle's MEM with 15% FBS and then transferred into amniotic growth media (AFM) — αMEM, Changs B&C, ES-FBS. Initial CD117$^+$ counts were: amniotic fluid (81%), fetal tissue (79%), 3-day-old (46%), 11-year-old (47%), 37-year-old (23%), and 96-year-old (0.5%). By the third passage in AFM, >85% of cells in each culture were CD117$^+$. The cultures were then differentiated into nervous, adipose, muscle, and hepatic cells. Remarkably, all samples differentiated into cells with the morphologic, metabolic, and staining characteristics of these tissue types. Undifferentiated cells were also positive for NANOG, a nuclear stem cell marker (85%/> in each sample). The above data indicates that all samples contain significant numbers of cells able to differentiate into all three germ layers. Our study suggests that skin fibroblast cultures may be a useful source of in vivo gene and/or autologist cell therapy.

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## Program/Abstract # 285

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## Program/Abstract # 286

### Interactors of Sox2 in embryonic stem cells

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Sox proteins form a large family of proteins, containing an HMG DNA binding domain and a transcriptional activator/repressor domain. They are important regulators of development and differentiation and are found throughout evolution.Sox2 is one of the key players in the network of proteins that maintains the self-renewal and pluripotency of embryonic stem (ES) cells. Sox2co-occupies many promoters together with pluripotency factors Oct4 and Nanog. It is likely that Sox2 regulates its target-genes with the help of interacting factors but besides Oct4 and Oct1, no such factors are currently known. We wanted to identify Sox2 interactors biochemically, by

affinity-purification of epitope-tagged Sox2 from ES cells. For this purpose, we have made ES cell clones that stably express Sox2 containing a combined biotinylation tag and a FLAG tag. Tagged Sox2 was purified from ES cell nuclear extracts with either anti-FLAG antibody beads or with streptavidin magnetic beads and its interacting proteins analyzed by mass spectrometry. Comparison between Sox2 pull-downs and pull-downs from control extracts, by mass spectrometry, suggests various ES cell self-renewal factors, lineage-specific transcriptions factors and a transport factor as putative Sox2-interacting proteins. We are currently studying the specific transport factor for Sox family proteins using import assays.

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**Program/Abstract # 288**
**Foxd3 is required for maintenance of multipotent neural crest progenitors**

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The neural crest (NC) is a heterogeneous pool of multipotent cells that gives rise to diverse derivatives including bones and cartilage of the face, smooth muscle of the cardiac outflow tract, and neurons and glia of the peripheral nervous system. The forkhead/winged-helix transcription factor Foxd3 regulates self-renewal and differentiation in both embryonic stem cells (ES cells) and trophoblast stem cells and is one of the earliest markers of the NC. Targeted NC-specific inactivation of Foxd3 in mice results in severe defects in NC derivatives including craniofacial defects, pharyngeal arch defects, and complete loss of the peripheral nervous system. *Foxd3* mutant embryos show increased cell death throughout the dorsal neural tube, consistent with loss of NC progenitors. Lineage labeling analysis of *Foxd3* mutant embryos demonstrates that vagal NC progenitors fail to migrate into the foregut, and the amount of NC is greatly reduced in the outflow tract of the heart. Surprisingly, this reduced amount of cardiac NC is able to mediate outflow tract septation and pharyngeal arch remodeling. These data suggest there are intrinsic differences between NC progenitor cell populations with respect to loss of Foxd3. In vitro analysis of differentiation in clonally derived mutant NC indicates a loss of multipotency and self-renewal of NC progenitors. These results demonstrate a global role for Foxd3 in NC maintenance along the anterior–posterior axis, and establish the requirement of Foxd3 in multipotent NC stem cell subpopulations.

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**Program/Abstract # 289**
**Nuclear interaction of homeodomain protein ZHX2 and ephrin-B1 in neural progenitor maintenance**

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Ephrin-B plays an important role in regulating neural progenitor self-renewal in the developing and adult brains. As one mechanism for this function, an RGS domain containing protein PDZ-RGS3 mediates the downstream signaling of ephrin-B and inhibits the Gα subunit signaling, thereby promoting the maintenance of neural progenitor cell state. Two recent studies have reported the proteolysis of ephrin-B into an intracellular carboxyl-terminal fragment, raising a possibility of a direct nuclear mechanism for ephrin-B function as documented in the Notch signaling pathway. Here we show that homeodomain protein ZHX2 selectively co-expresses with ephrin-B1 in neural progenitor cells of the developing cerebral cortex and recruits the cytoplasmic domain of ephrin-B1 into the nucleus. Binding of ephrin-B1 makes ZHX2 a stronger repressor of transcription. In the cerebral cortex, converting ZHX2 into a constitutive transcriptional activator causes differentiation of cortical neural progenitors, while co-expression of ZHX2 and ephrin-B1 cytoplasmic domain prevents differentiation. This study reveals a novel nuclear function of ephrin-B and identifies ZHX2 as a cooperative nuclear factor with ephrin-B in the maintenance of neural progenitor cells.

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**Program/Abstract # 290**
**Differential gene expression profile in comparative microarray between olfactory ensheathing cells and striatal embryonic stem cell**

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In mammals, CNS regenerates spontaneously in certain regions, as olfactory bulb (OB) mainly due to the presence of a type of macroglia that promotes growth; aldynoglia. The prototype of this glia is the ensheating cells (EC) which functional expression profile are not yet fully understood. EC can be continuously generated from local precursor cells within the OB. Multipotent neural precursors (MNP) have been isolated from the embryonic and adult brain, maintained in culture and they are capable of proliferating and differentiating. To compare the expression profile among EC and MNP, we hybridized 5K microarrays and analyze it by Genarise software. Genes highly expressed by the EC when compared to the MNP were mainly expressed in glial cells and in CNS, and eight of them were expressed in the OB in vivo, indicating that the gene expression profile obtained here for the EC, corresponds well with their phenotype. RT-PCR confirms that S100a6, Mtmr2 and Col5a, were highly expressed by EC. The expression profiles support a closer relationship of EC to Schwann cells and astrocytes than to oligodendrocytes. In addition, we found that 58 genes were strongly expressed in MNP. By grouped analysis they correspond to an undifferentiated cell profile, with several genes previously shown to be expressed by stem cells, which validate the expression profile. Pou3f3 and Ckb, were more strongly expressed in MNP than in EC. The results of these analyses increased the number of characteristic genes of these two particular cell phenotypes.

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